Utilisation of Silane Modified Surfaces to Control the Osteogenic Differentiation of Human Mesenchymal Stem Cells

J.M Curran, R. Chen & J.A. Hunt

UK Centre for Tissue Engineering, Department of Clinical Engineering, The University of Liverpool

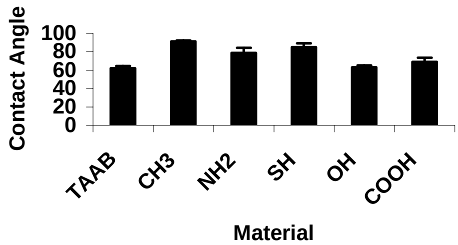
INTRODUCTION: The ability to control, or influence, the generic plasticity of bone marrow derived mesenchymal stem cells (MSC), is an area that is still relatively unexplored. The aim of this study was to establish how changes in surface chemistry (characterised silane modified surfaces), both with and without the appropriate biological stimuli, affected the behaviour and osteogenic potential of human MSC in vitro over a 28 day time period.

METHODS: Glass coverslips (13mm diameter, Borosilicate Glass Co. UK) were dipped into 5% NaOH solution for 1 hour followed by concentrated HNO_3 for 1 hour. All the coverslips were then rinsed with ultra pure water and with 100% ethanol, dried at 120^{o}C, and stored in a vacuum desiccator prior to the introduction of different functional groups on the surface. TAAB glass was used as a control surface. –CH3, -NH2, -SH, -OH and –COOH groups were introduced using previously described methods[1] and characterized using water contact angle measurements.

Human MSC (Biowhittaker UK), were re-suspended to a concentration of 5x10^4 cells/ml, and cultured in contact with the samples for 1,7, 14, 21 and 28 days in both basal MSC media (Biowhittaker UK) and osteogenic supplemented media (DMEM + 10% FCS, 100mM ascorbate-2-phosphate, 100nM dexamethasone and 10mM β-glycerphosphate). Viable cell adhesion was quantified using a commercially available lactate dehydrogenase assay (LDH, Promega UK). Real time PCR was used to evaluate the expression of β-actin, ornithine decarboxylase, osteocalcin, osteopontin, osteonectin, collagen I and CBFA-1. Cellular expression of collagen I, osteocalcin, CBFA1 and calcified extra-cellular matrix was qualitatively evaluated by fluorescent immunohistochemistry and von Kossa staining.

RESULTS: Dynamic contact angle measurements using water as the solvent for glass and silane modified surfaces are shown in Figure 1.
The surface energy results supported the contact angle results. The TAAB, -OH, -COOH have the highest surface energies with values of 53.39, 52.89 and 49.51mJ/m^2, respectively. The -NH_2 and -SH had lower values of 43.52 and 39.60mJ/m^2 respectively, and the -CH_3 had the lowest value of 35.53mJ/m^2.

Fig. 1: Water contact angle measurements



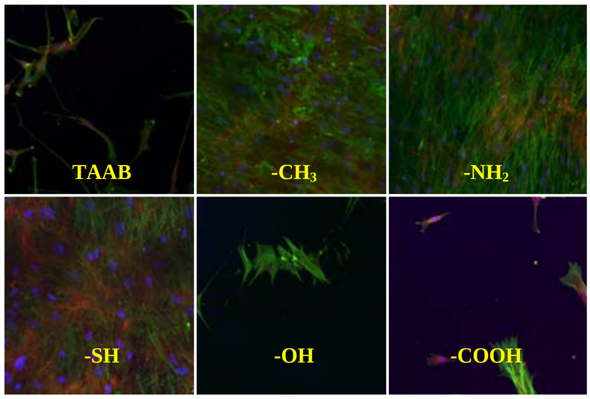


Fig. 2. 14 day collagen I expression (red) in osteogenic supplemented medium.

DISCUSSION & CONCLUSIONS: Changes in surface chemistry did affect the behaviour of the MSC and subsequent osteogenic differentiation in vitro. –TAAB, -OH and –COOH surfaces did not maintain cell adhesion under osteogenic conditions. –CH_3, -SH and –NH_2 provided a favourable surface for osteogenic differentiation in all culture conditions.

REFERENCES: [1] *Filippini P et al (2001) J Biomed Mater Res 55:338-349*